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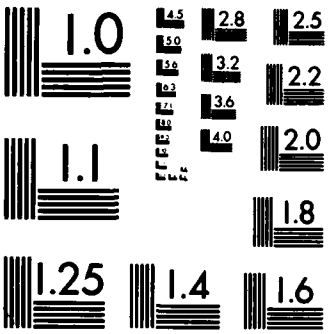

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Crab zoeae (Rhithropanopeus harrisii) were exposed to water soluble fractions of jet fuel (JP-5) for the first 5 days or for the duration of zoeal development (11 to 14 days). Short term exposure or continious exposure to low concentrations of petroleum hydrocarbons caused no increase in mortality or changes in development rate and increased megalopal weight was characteristic of such groups. This phenomenon, termed, "hormesis" is probably a generalized aspect of environmental stress etiology but has seldom been reported as such.

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Hormesis: A Response to Low Environmental Concentrations of Petroleum Hydrocarbons

Roy B. Laughlin, Jr., James Ng, and Harold E. Guard

Hormesis: A Response to Low Environmental Concentrations of Petroleum Hydrocarbons

Abstract. *Crab zoeae* (Rhithropanopeus harrisii) *were exposed to water-soluble fractions of jet fuel (JP5) for the first 5 days or for the duration of zoeal development (11 to 14 days). Short-term exposure or continuous exposure to low concentrations of petroleum hydrocarbons caused no increase in mortality or changes in development rate, and increased megalopal weight was characteristic of such groups. This phenomenon, termed "hormesis," is probably a generalized aspect of environmental stress etiology but has seldom been reported as such.*

Most experiments dealing with the environmental effects of oil pollution have examined short-term determinations of acute toxicity (*1*, *2*) or long-term effects on growth, development rate, or reproduction (*3*). The results of these experiments have often been used to assess the damage resulting from episodic events such as spectacular oil-tanker wrecks or offshore oil-well blowouts. Although these incidents receive a great deal of public scrutiny, they make relatively small contributions to the total petroleum hydrocarbon burden entering the marine environment each year (*4*). In many oil spill incidents, hydrocarbon concentrations return to base-line levels after the surface slick dissipates, usually several days to weeks after the event (*5*). Therefore, the question often arises of whether these short-term exposures to pollutants cause lasting harm to affected individuals after the exposure ends (*6*).

To investigate the recovery process after exposure to petroleum hydrocarbons, we exposed zoeae larvae of the mud crab, *Rhithropanopeus harrisii*, to water-soluble fractions (WSF) of jet fuel (JP5) for either the first 5 days or for the duration of zoeal development (11 to 14 days). There are four zoeal stages followed by metamorphosis to the megalops stage. The first zoeal stage, lasting 3 to 4 days, is the most sensitive to petroleum hydrocarbon exposure (*7*). The response in two salinities, 5 and 15 per mil (*8*), was determined for a control and a range of WSF concentrations from 10 to 100 percent of the original solution (*9*). Zoeae were reared in 8-cm (diameter) finger bowls containing 50 ml of JP5 WSF with ten zoeae per bowl; there were three bowls per hatch to give 30 larvae from each female. Each day the zoeae were censused for living and dead animals. They were moved to clean bowls containing freshly prepared WSF or clean artificial seawater, as appropriate, and given freshly hatched *Artemia* nauplii as food. The indices of sublethal stress that we used were development rate and the weight of the megalops (*10*).

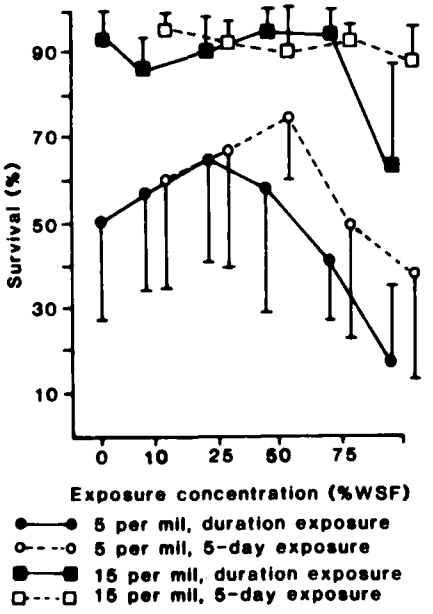

Fig. 1. Survival of zoeal mud crabs, *Rhithropanopeus harrisii*, exposed to JP5 WSF. Survival in 15 per mil salinity was much higher than in 5 per mil salinity. Lowest survival occurred in high, continuous WSF exposure in low salinities. For the purpose of clarity in this and subsequent figures, points for 5-day and duration exposure groups are slightly displaced relative to each other over the WSF designations. Vertical lines are 1 standard deviation of the mean.

All the factors tested, salinity, WSF concentration, and the length of exposure, influenced the survival of the zoeae (Fig. 1). Zoeae in a salinity of 5 per mil showed markedly decreased survival compared to those in 15 per mil. Exposure to JP5 WSF caused much greater toxicity for zoeae in 5 per mil than in 15 per mil salinity, with maximum toxicity within each salinity occurring in groups exposed for the duration of zoeal development to 100 percent WSF. Under most 5-day exposure regimes, there was no effect of JP5 WSF on survival; however, there appeared to be enhanced survival in midrange and a decrease at maximum JP5 WSF concentrations in 5 per mil salinity. An analysis of variance showed that the effects of salinity and WSF concentration, but not the length of exposure, were significant ($P < .01$) (*11*). By comparison, the JP5 WSF are much less toxic to developing crustaceans than those of other refined oils tested (*7*, *12*) and would not be classified as acutely toxic (≥ 90 percent mortality) as defined by Epifanio (*13*).

Sublethal effects of JP5 WSF exposure were evident in terms of changes in both development rate and the weight of the megalops. At each salinity, development rates were approximately the same, with a slight tendency for those in 15 per mil salinity to require longer at higher WSF doses to complete development (Fig. 2). Controls required 11.5 to 12 days to reach metamorphosis. In general, there was a dose-dependent increase in development time which was markedly greater in the group in 15 per mil salinity. Notably, at low JP5 WSF levels and low salinity the exposure duration exerted the least effect. Analysis of variance showed that all the factors tested contributed significantly to the variance ($P < .01$) (*10*).

As shown here, megalopal dry weight is a sensitive indicator of sublethal stress, the degree of growth inhibition being proportional to toxicity. Larvae grown in low salinities showed the poorest performance overall (Fig. 3). The difference between the "duration" and 5-day groups is noteworthy. At each salinity, the line connecting the duration and 5-day groups tends to diverge with increasing WSF concentration. Differences between the two groups in each salinity are statistically significant ($P < .001$). In every case, mean megalopal weights for 5-day groups were equal to or in

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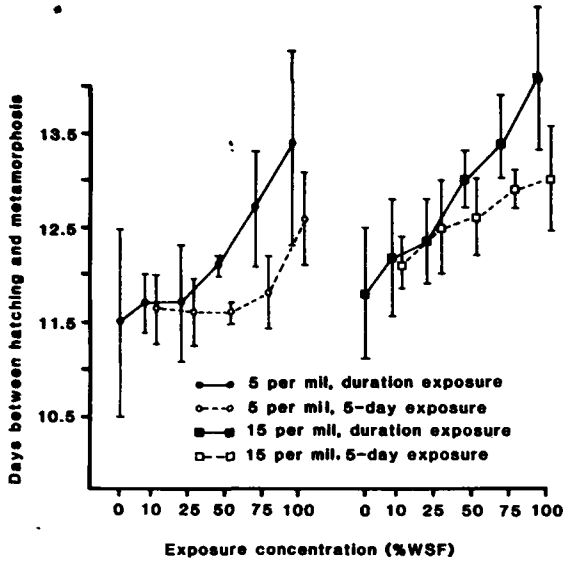

Fig. 2. Development rate of mud crabs, *Rhithropanopeus harrisii*, exposed to JP5 WSF. Zoeae exposed during only the first 5 days showed increased development rates as compared to controls, the decrement increasing with WSF exposure concentration. Vertical lines are 1 standard deviation of the mean.

some cases slightly larger than (that is, 15 per mil, 10 percent WSF) control values. If growth inhibition occurs to an equal extent during WSF exposure, increased growth rates after cessation of exposure would account for the similarity between controls and those exposed for 5 days. In continuous exposure to WSF concentrations greater than 25 percent growth inhibition was significant. Differences in growth have been noted for *R. harrisii* exposed to low salinities or to petroleum-derived aromatic hydrocarbons, or both, and have been attributed in part to increased respiratory loss or carbon (*7*, *12*).

Since JP5 WSF are not acutely toxic to *R. harrisii* zoeae under the conditions used here, organismic responses may be termed sublethal. The data show that exposure to low aqueous hydrocarbon concentrations, either in low doses or for relatively short periods, are followed by enhanced growth rates. The occurrence of greater growth rates in WSF-exposed megalops shows that we are observing an actual effect, not the absence of one due to low toxicity. The apparent enhancement of a physiological process by low toxicant doses is well known in pharmacology and toxicology. It is called the Arndt-Schult effect, hormesis (*14*), or "sufficient challenge" (*15*). Mechanistically, it has been attributed to "transient overcorrections by control mechanisms to inhibitory challenges well within its capacity to counteract" (*14*, p. 480). Data consistent with an interpretation of hormesis during exposure to low concentrations of environmental pollutants have been reported in widely diverse animal groups, for example, coelenterates (*14*), polychaetes (*16*), and fishes (*17*). Only in (*14*) has hormesis been considered a functional part of stress etiology. In the future, enhancements of sublethal stress indices noted during long-term bioassays should be clearly identified as such.

To our knowledge, no other study has examined the interaction between suboptimal physical factors, duration of pollutant exposure, and hormesis. It appears that, in sublethal doses, pollutants exert their influence within the context of a major stressor's effect. In this case, low salinity was a major stressor leading to a decrease in megalopal weight, survival, and development rate. However, its effect was not so severe that hydrocarbon exposure failed to elicit hormesis. Furthermore, differences in the hormesis response at the two salinities demonstrate that the linear "dose-response" models often suggested to explain toxicological responses would be inadequate here. Nonlinear simultation has been suggested as a more profitable approach to these problems (*18*).

On a more practical level, these experiments suggest an organismic resilience to episodic oil-spill incidents. It is likely that many marine organisms have compensatory physiological strategies that enable them to tolerate low concentrations of pollutants or exposure for a short duration, whereas long-term hydrocarbon inputs are probably the most environmentally hazardous.

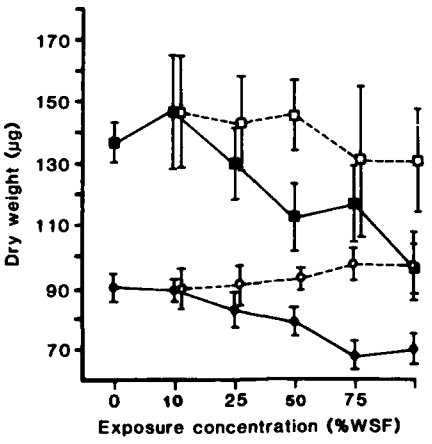

Fig. 3. Dry weights of mud crab, *Rhithropanopeus harrisii*, megalops exposed as zoeae to JP5 WSF. Those in 5 per mil salinity weighed much less than the ones in 15 per mil salinity. Continuous exposure to WSF during zoeal development caused a dose-dependent weight decline in both salinities. Groups exposed for 5 days showed enhanced growth in low WSF doses and in low salinities. Vertical lines are 1 standard deviation of the mean.

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8. Ovigerous *Rhithropanopeus harrisii* were collected in Sykes Creek, Brevard County, Florida, brought to California by air, and maintained in the laboratory in 15 per mil salinity at room temperature until the larvae hatched. Zoeae from each female were divided into two groups. The first was exposed to JP5 WSF in 15 per mil salinity, the hatching salinity. The second group was in seawater diluted to 5 per mil salinity by the slow addition of deionized water during a 5-hour acclimation period. This is enough time for the animals to come to osmotic equilibrium [F. A. Kalber, Jr., and J. D. Costlow, Jr., *Am. Zool.* **6**, 221 (1966)]. As a result of salinity acclimation, there was about 7 hours maximum difference in initiation time for zoeae in 15 and 5 per mil salin-

ity. However, in all groups exposure to JP5 WSF began during the first day of zoeal development. The progeny from three females were used for each group. The larvae were reared in two incubators set at a nominal temperature of 25°C. (Differences in development rate suggest that the incubator containing the groups at 15 per mil salinity either had wider variation or maintained slightly lower temperatures than the incubator containing the groups at 5 per mil salinity.)

9. Water-soluble fractions of JP5 were made by gently stirring one part JP5 over nine parts seawater of the appropriate salinity at room temperature (*1*). We ensured a uniform stirring rate in each bottle by using a stirring table with multiple heads revolving at identical speeds. Because higher salinity causes a decrease in the time-dependent dissolution of hydrocarbons, the 15 per mil WSF were stirred for 3 to 4 hours longer each day to more closely equalize exposure concentrations. Ultraviolet spectrophotometry [J. M. Neff and J. W. Anderson, *Bull. Environ. Contam. Toxicol.* **14**, 122 (1975)] of the daily WSF preparations gave total aromatic hydrocarbon values of 2.62 ± 0.15 parts per million (ppm) (N = 17) in 5 per mil salinity and 2.70 ± 0.16 ppm (N = 18) in 15 per mil salinity, expressed as tetralin equivalents. Tetralin is a major ultraviolet-absorbing constituent of JP5 WSF. Gas chromatographic analysis indicated that alkyl-substituted monocyclic aromatics predominate. Fortuitously, concentrations of total aromatics determined here by ultraviolet spectrophotometry are similar to those determined by gas-liquid chromatography.
10. Megalops were rinsed briefly in tap water to remove adsorbed sea salts, dried for at least 2 days at 60°C, and weighed to the nearest 0.1 μg on a microbalance (Cahn 21). Generally, 15 megalops were weighed and three means determined as replicates for further statistical analysis. In several instances when fewer than three megalops per mean were available, the number of replicates was reduced from three, as generally used. Figure 3 shows the means across all hatches.
11. Percentage of survival was determined for each hatch to give three replicates per factor combination and transformed to the arc sin $x^{1/2}$ [R. G. D. Steele and J. H. Torrie, *Principles and Procedures of Statistics* (McGraw-Hill, New York, 1960)]. We performed statistical tests, using the "statistical package for the social sciences" computer programs [N. H. Nie, C. H. Hull, J. G. Jenkins, K. Steinbrenner, D. H. Bent, *Statistical Package for the Social Sciences* (McGraw-Hill, New York, 1975)]. A regression analysis approach, as explained in the manual, was used. Data for the development rate and megalopal dry weights were treated similarly, except that they required no transformation.
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